Section A -Research paper

FORMULATION AND EVALUATION OF ALCOHOL BASED HERBAL HAND SANITIZER

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Abstract:

The aim of the present work was to prepare an alcohol based herbal hand sanitizer. This sanitizer was prepared by using herbal oils such as eucalyptus oil, lemongrass oil, clove oil and aloe vera gel including other excipients. The sanitizer was formulated by the dispersion method. The excipients which were used included denatured alcohol, Carbopol 940, polysorbate 20, glycerine, triethanolamine and preservatives. This study was aimed to prepare an alcohol based herbal hand sanitizer for antimicrobial purpose. The pH of sanitizer was found to be between 6.56 to 6.67. The viscosity of sanitizer was found to be between 376 to 838. The spread ability was good and found in range between 6.33 to 7.16. The drug content of Eucalyptus Oil in formulations was found to be 89.8% in F1, 93% in F2 and 97.4% in F3 and the drug content of Lemongrass Oil in formulations was found to be 87% in F1, 88% in F2 and 90% in F3. In-vitro drug release study was found to be 52.6% in F1, 35.2% in F2 and 62.65 in F3. The Anti-microbial study of the formulation was done against the standard drug (Streptomycin) and found to be effect and the Stability of the formulations was found to be stable upon storage for 3 months. All these studies have revealed that the formulation F3 is best because it has better pH, Viscosity, spread ability, Drug content, In-vitro drug release study, Anti-microbial study and Stability.

Keywords: Denatured, dispersion, in-vitro, preservatives, anti-microbial.

1. Introduction:

Skin is the most exposed part of the body to the daylight, environmental pollution and also to some protection towards the pathogens. The most common skin problems are eczema (atopic dermatitis), warts, acne, psoriasis, rashes, allergic reaction etc. To protect the skin from harmful micro-organisms and to prevent spreading of many pores and skin infection. Hand cleaning is certainly a vital precaution. Hand sanitizers are one of the most commonly used hygienic products to prevent and remove germs, viruses and bacteria in our body, especially in our hands. It is more handy and

convenient, unlike other products that are used for different cleaning purposes. There are several preparations of hand sanitizers are available which include gel, foam, and liquid solution. Typically, denatured alcohol (ethanol) serves as the active ingredient in hand sanitizers. Inactive ingredients include polyacrylic acid for thickening alcohol gel, glycerin for liquid rubs, polypropylene glycol, and plant essential oils. In comparison to soap, alcohol-based hand sanitizer is far more effective in killing the majority of microorganisms and doesn't cause as much hand drying. The range for the alcohol content is 60-81%, with 62% being the most common number. Hand sanitizers with alcohol destroy the majority of bacteria, fungus, and certain viruses as well. Alcohol-based hand sanitizers (ABHS) are an alternative to hand washing with cleaning soap that doesn't need water. It has been found to enhance hand hygiene compliance and to considerably reduce the rate of infection, in healthcare settings. Many laboratory studies have checked and verified that ABHS can decrease bacterial test organisms, for example, Escherichia coli and Staphylococcus aureus with greater magnitudes than cleanser (soap) and water. ABHS effectiveness has additionally been tried to be equal or more than hand washes with soap at removing the bacterial infection in field settings among veterinary staff workers in Canada, livestock handlers in the US, and mothers visiting a health clinic in Tanzania [1-3]. Historically, plants have provided an excellent source of anti-infective agents. Plant extract has a potential as antimicrobial compounds against several pathogenic micro-organisms which cause infectious disease and resistance towards synthetic drugs. The advantage of using herbal essential oils are that they are cheap, easily available and having less side effects in comparison to chemical products. Traditional healers have long used the plant in infectious situations. Because plants have secondary metabolites which contain tannins, alkaloids, terpenoids and flavonoids etc. That has been determined to possess in-vitro antimicrobial properties. Thinking about this ultimatum; an effort has been created to screen classical literature for the herbs with anti-microbial properties and it's been determined that Eucalyptus oil, Lemongrass oil, Clove oil, and Aloe Vera gel holds that antibacterial potency. Along these lines, we planned to formulate and evaluate alcohol-based herbal hand sanitizer including alcoholic extracts mixture of these astonishing herbs utilizing other reasonable excipients; which can be used as ready to use alcohol-based herbal hand sanitizer. Hand sanitizers would now be able to be found in the entrances to nursing homes and hospitals and in lots of public washrooms. We all realize the significance of right hand-washing in decreasing harmful germs transmission [4-6].

Benefits of Using Herbal Hand Sanitizer:

Ease of availability: Herbs are easily available in rural as well as urban areas, so they can be easily used by anyone.

Cheap: Cost of the herbal plants is less as compared to chemical ingredients used in synthetic hand sanitizers.

Increased efficiency: Herbal hand sanitizers are more efficient in promoting hand hygiene.

Less side effects: Herbal hand sanitizers have less side effects than other hand sanitizers.[7]

2. Materials and methods:

Materials:

The plant materials were collected from the medicinal garden of Azad Institute of Pharmacy and Research Institute located in Lucknow. The taxonomical identification and authentication was done by Department of Horticulture of Babasaheb Bhimrao Ambedkar University, Lucknow. The hand

sanitizer was prepared from the volatile oil of *Eucalyptus globulus*, *Cymbopogon citratus* and *Syzygium aromaticum.*[8] The volatile oil of each plant was obtained by hydro distillation method.

Methods:

Volatile Oil Extraction Procedure:[9]

Sample Preparation of Eucalyptus Leaves:

Fresh leaves of Eucalyptus were collected and washed with tap water to remove the dust and dirt over its surface. After draining free water on the surface of the leaves than weight 100 gms of leaves and chopped into small pieces with a sharp-edged knife.

Sample Preparation of Lemongrass:

Fresh lemongrass was collected and washed with tap water to remove the dust and dirt over its surface. After draining free water on the surface of the lemongrass stem, then weight 100 gms of leaves and chopped into small pieces with a sharp-edged knife.

Hydro Distillation Procedure:[10]

- First of all purified water was taken into a round bottom flask.
- Then cut leaves samples were placed in round bottom flask for distillation.
- After this the round bottom flask attached to the connecting pipe of the condenser and a mercury thermometer was inserted in hole of the flask so as to touch the top of the leaves bed.
- Then heating mental was switched on and kept in a pre-selected position to obtained desired rate of heating.
- Observations of cumulative extracted oil volume, temperature of extraction chamber and energy meter reading were recorded at every 30 minutes time interval. The weight of hydrosol, spent eucalyptus leaves and lemongrass and left over water in the extractor was also recorded at the end of each distillation test.
- At the end of process extracted eucalyptus and lemongrass oil were stored in refrigerator below 4˚C.

Extraction of Clove Oil:

One hundred and fifty grams (150gm) of whole buds and ground clove were separately hydro distilled with 300ml of water for 3 hours using all glass Clevenger apparatus. It was heated at 100˚C by heating mantle; the essential oils were collected, dried over anhydrous sodium sulphate and stored in refrigerator below 4˚C.[11]

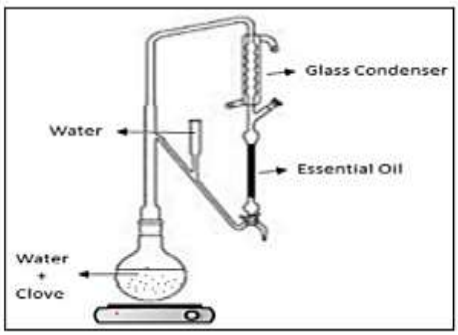

Figure 5 Clevenger hydro distillation apparatus.

Procedure for formulation of Hand Sanitizer:

The alcohol-based herbal hand sanitizer was made by continuously stirring Carbopol 940 into distilled water. The product was set aside for 24 hours after homogeneous mixing. Denatured alcohol, polysorbate 20, glycerin, and all of the extracts had been combined with the aqueous medium. Finally, to create a homogeneous product, 0.70% of Triethanolamine and 0.25% of each of the preservatives methyl and propyl paraben were added. Prepared product was stored in airtight High-density polyethylene (HDPE) containers. The formulated hand sanitizer was used initially for physical evaluation and then for screening for antimicrobial sensitivity.[12,13,14]

Table 1: Formulation Table

S. No.	INGREDIENTS	QUANTITIES		
		F1	F2	F3
1.	Deionized water	8.94 ml	8.34 ml	7.74 ml
2.	Denatured alcohol	18.6 ml	18.6 ml	18.6 ml
3.	Eucalyptus oil	0.30 ml	0.45 ml	0.60 ml
4.	Lemongrass oil	0.30 ml	0.45 ml	0.60 ml
5.	Clove oil	0.15 ml	0.30 ml	0.45 ml
6.	Aloe vera gel	0.30 ml	0.45 ml	0.60 ml
7.	Carbopol 940	0.21 gm.	0.21 gm.	0.21 gm.
8.	Glycerin	0.69 ml	0.69 ml	0.69 ml
9.	Polysorbate 20	0.15 ml	0.15 ml	0.15 ml
10.	Tri ethanol amine	0.21 ml	0.21 ml	0.21 ml
11.	Preservative (Methyl + Propyl Paraben)	0.15 gm.	0.15 gm.	0.15 gm.
Total Weight		**30ml**	**30ml**	**30ml**

Evaluation parameters:[15]

1. Visual appearance:

The prepared sanitizer was visually inspected for clarity, colour and transparency and presence of any particles.

The smear of sanitizers were prepared on the glass side and observed under the microscope for the presence of any particle or grittiness.

2. Physical Evaluation:[16]

a) pH:

Using a digital pH metre, the pH of an alcohol-based herbal hand sanitizer formulation was evaluated. In 100 millilitres of distilled water, 1 gram of gel was dispersed, then left to stand for two hours. The measurement of pH of each formulation should be done in triplicate and average values were calculated.

b) Viscosity:

Using a brook field viscometer, the viscosity of an alcohol-based herbal hand sanitizer composition was assessed at 37 °C. For determining viscosity, a Brook field viscometer connected to a T-bar spindle (S-94) had been utilized. A 10ml beaker containing 5gm of solution was filled, and the spindle was then lowered perpendicularly while being careful to keep the spindle away from the beaker's bottom. To produce a torque larger than 50%, the spindle was turned at speeds of 50, 60, and 100 rpm. 60 seconds after the measurement was taken, measurements were taken.

c) Spreadability:

The spreadability of an alcohol-based herbal hand sanitizer was tested using a laboratory-made device with two glass slides, the top one of which was linked to a balance by a hook and the bottom one glued to a wooden plate.

The Spreadability of sanitizer was calculated by using formula:

$$S = m \times l/t \text{ (gram cm/sec)}$$

Where,

m = wt. Tied to upper slide

L = length of glass slides

T = time taken to separate the slides

d) Determination of λ_{max}:

The λ_{max} of different essential oils was determined by the following method:

- Weigh accurately 100mg of oil and then transfer it in a 100ml volumetric flask.
- Add sufficient quantity of ethanol and shake the solution thoroughly to dissolve the essential oil.
- Finally make up the volume to 100ml with the help of solvent (ethanol).
- Put the dilution in cuvette.
- Set the spectrophotometer wavelength to 200 to 400nm with nothing in the spectrophotometer light path (or cuvette containing distilled water) set the reference level.
- Place the cuvette containing the prepared dilution in the sample compartment. Record the absorbance.
- Repeat steps 4 to 5 at the same wavelength and record absorbance.
- Plot the results as absorbance against wavelength

e) Drug content estimation:

The drug content of the hand sanitizer was determined by dissolving an accurately weighed quantity 1000mg of sample in 100ml of solvent (Ethanol).

The solutions were kept for shaking for 4 hours and kept for 6 hours for complete dissolution of the formulations. Then the solutions were filtered and dilutions were made and solutions were subjected to the spectrophotometer analysis. The drug content was estimated by using UV/Visible spectrophotometer at 260nm for eucalyptus oil, 245nm lemongrass oil. [17]

The drug content was calculated from the linear regression equation obtained from the calibration data.

f) Preparation of dialysis membrane for in-vitro release study:

Drug release study was carried out using treated dialysis membrane. The membrane was treated before carrying out the release study. The membrane was washed in running water for 4-5hrs to remove the glycerine and then treated with 0.3% w/v solution of sodium sulphide at 80˚C for 1-2 minute to remove sulphur compounds. Subsequently, the membrane was washed with hot water at 60˚C for 2 minutes followed by acidification with 0.2% v/v solution of sulphuric acid. Finally, the membrane was rinsed with hot water to remove the acid contents. The treated membrane was stored in the diffusion medium and refrigerated till use. The membrane was washed with distilled water prior to use for in-vitro study.[18]

g) In-vitro release study:

Franz diffusion cell (with effective diffusion area 3.14cm^2 and 15.5ml cell volume) was used for the drug release studies. Alcohol Based Herbal hand sanitizer (200mg) was applied onto the surface of membrane evenly. The membrane was clamped between the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with phosphate buffer (7.4) different sample was withdrawn through the sampling port and replaced with fresh buffer at 0, 0.5, 1, 2, 4, 6, 8 and 24hrs. After suitable dilutions, the absorbance was measured spectrophotometrically at 260nm, 245nm, 282nm and 263nm.The procedure was performed in triplicate and the average percentage of the drug released was calculated.

h) Antimicrobial Study:

The screening of anti-microbial efficacy of the formulated alcohol based herbal hand sanitizer was aseptically performed on Escherichia coli, Staphylococcus aureus, and Bacillus subtilis by using agar well disk diffusion technique. A well was prepared in the plates (containing 15ml of Muller Hinton agar medium) with the help of a cork-borer (0.85cm) 100µl of the test compound (alcohol based herbal hand sanitizer) added in the each well. The Standard antibiotic disk of Streptomycin was used as a standard. The plates were incubated overnight at 37˚C for 24 hrs. Efficiency of alcohol based herbal hand sanitizer was determined by measuring the diameter of zone of inhibition.[19,20]

i) Stability Studies:

The prepared alcohol based herbal hand sanitizer containing essential oils were stored away from light in collapsible aluminium tube in triplicate, and stored at refrigerated temperature (2-8˚C), room temperature (25 ± 2˚C) and oven temperature (45˚C) for 3 months. After storage, the samples are tested for their physical appearance, pH, viscosity, drug release.

3. Result and discussion:

3.1 Visual appearance:

3.1.1 Colour: Colourless to light yellow.

3.1.2 Odour: Aromatic and characteristics.

3.2 pH determination:

Table 3.1 pH determination of Alcoholic herbal hand sanitizer

S. No	FORMULATIONS	pH Values
1.	F1	6.65
2.	F2	6.56
3.	F3	6.67

The pH of each sanitizer formulation after dispersion in distilled water was noted and the result were taken. Showing in Table 3.1. The pH of the alcohol based herbal hand sanitizer was found to be between 6.56 to 6.67 which was well within the normal pH range of 5.5-7, Hence the prepared herbal hand sanitizer can be applied to the hand.

3.3 Viscosity:

Table 3.2 Viscosity of Alcoholic herbal hand sanitizer

S. No.	FORMULATION	VISCOSITY (cps)		
		50 rpm	60 rpm	100 Rpm
1.	F1	376	434	756
2.	F2	389	458	783
3.	F3	416	483	838

The viscosity of alcohol based herbal hand sanitizer formulation was determined at 37˚C using a Brook field viscometer. The viscosity values ranged from 376 cps to 838 cps, shown in the Table 3.2.

3.4 Spread-ability:

Table 3.3 Spread ability of Alcoholic herbal hand sanitizer

S. No.	FORMULATION	SPREADABILITY (g-cm/sec)
1.	F1	6.33
2.	F2	6.54
3.	F3	7.16

The Spreadability of the sanitizer was found to be in the range of 6.33 to 7.16 g-cm/sec confirming that the sanitizer may spread smoothly and uniformly shown in table. 3.3.

3.5 Drug Calibration Curve:

3.5.1 Calibration Curve for Eucalyptus Essential Oil (Formulation 1):

3.5.2 λ_{max} determination:

The λ_{max} was determined by using the U.V. Spectrophotometer. The λ_{max} was found to be 260nm.

3.5.3 Construction of calibration curve:

The calibration curve of Eucalyptus Oil was prepared in ethanol. The obtained calibration curves of eucalyptus oil obeyed the Beer-Lambert's law in the selected concentration in Table 3.4.

Table 3.4 Calibration Curve of Eucalyptus Oil (Formulation 1)

S. No.	Concentration (µg/ml)	Absorbance
1.	2	0.183
2.	4	0.377
3.	6	0.579
4.	8	0.782
5.	10	0.986

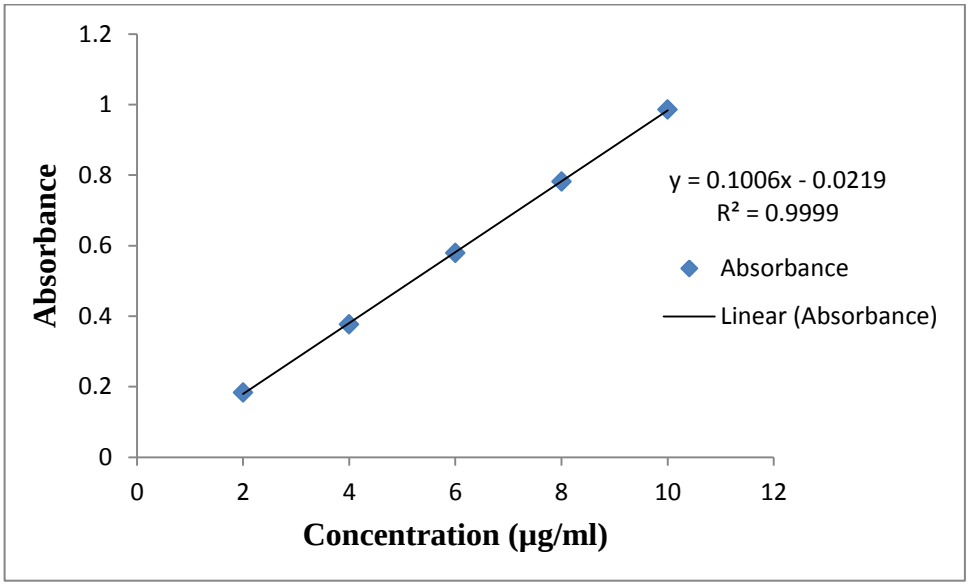

Fig 3.4: Standard Curve of Eucalyptus oil at λ 260nm (Formulation 1)

3.5.4 Calibration Curve for Eucalyptus Essential Oil (Formulation 2):

3.5.5 λ_{max} determination:

The λ_{max} was determined by using the U.V. Spectrophotometer. The λ_{max} was found to be 260nm.

3.5.6 Construction of calibration curve:

The calibration curve of Eucalyptus Oil was prepared in ethanol. The obtained calibration curves of Eucalyptus oil obeyed the Beer-Lambert's law in the selected concentration.

Table 3.5 Calibration Curve of Eucalyptus Oil (Formulation 2)

S. No.	Concentration (µg/ml)	Absorbance
1.	2	0.188
2.	4	0.386
3.	6	0.587
4.	8	0.784
5.	10	0.992

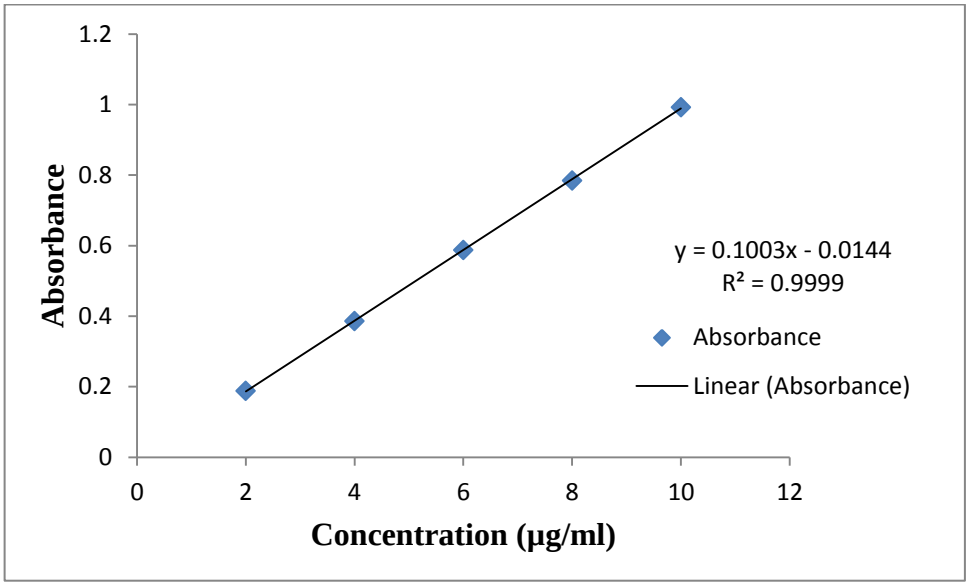

Fig 3.5 Standard Curve of Eucalyptus oil at λ 260nm (Formulation 2)

3.5.7 Calibration Curve for Eucalyptus Essential Oil (Formulation 3):

3.5.8 λ_{max} determination:

The λ_{max} was determined by using the U.V. Spectrophotometer. The λ_{max} was found to be 260nm.

3.5.9 Construction of calibration curve:

The calibration curve of Eucalyptus Oil was prepared in ethanol. The obtained calibration curves of Eucalyptus oil obeyed the Beer-Lambert's law in the selected concentration.

Table 3.6 Calibration Curve of Eucalyptus Oil (Formulation 3)

S. No.	Concentration (µg/ml)	Absorbance
1.	2	0.197
2.	4	0.398
3.	6	0.596
4.	8	0.813
5.	10	0.998

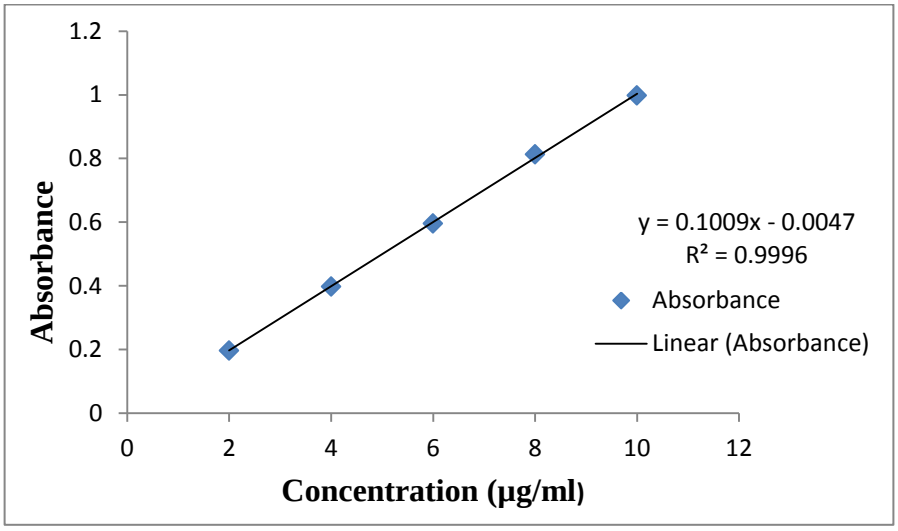

Fig 3.6 Standard Curve of Eucalyptus oil at λ 260nm (Formulation 3)

3.5.10 Calibration Curve for Lemongrass Essential Oil (Formulation 1):

3.5.11 λ_{max} determination:

The λ_{max} was determined by using the U.V. Spectrophotometer. The λ_{max} was found to be 245nm.

3.5.12 Construction of calibration curve:

The calibration curve of Lemongrass Essential Oil was prepared in ethanol. The obtained calibration curves of lemongrass essential oil obeyed the Beer-Lambert's law in the selected concentration.

Table 3.7 Calibration Curve of Lemongrass Essential Oil (Formulation 1)

S. No.	Concentration (µg/ml)	Absorbance
1.	2	0.175
2.	4	0.362
3.	6	0.574
4.	8	0.768
5.	10	0.983

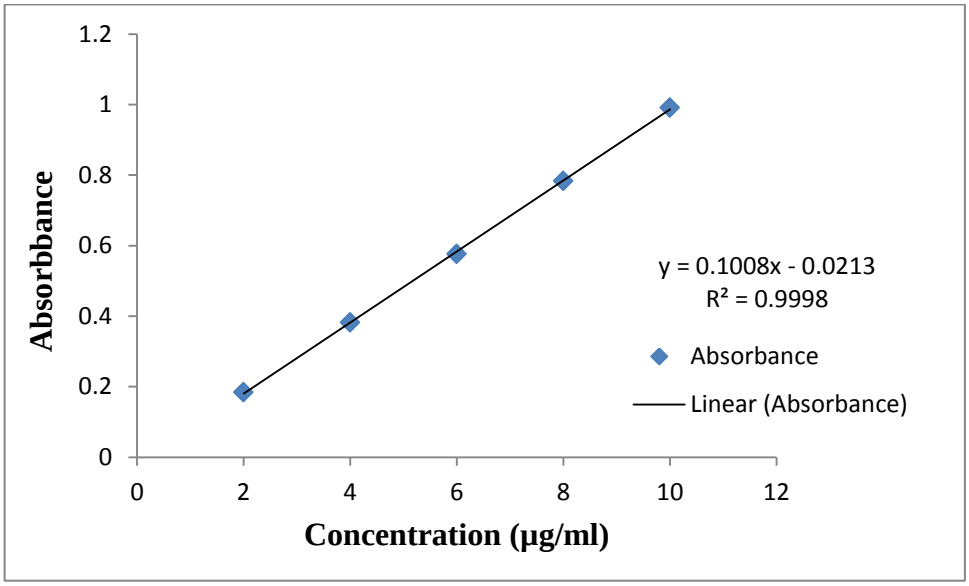

Fig 3.7 Standard Curve of Lemongrass Essential Oil at λ 245nm (Formulation 1)

3.5.13 Calibration Curve for Lemongrass Essential Oil (Formulation 2):

3.5.14 λ_{max} determination:

The λ_{max} was determined by using the U.V. Spectrophotometer. The λ_{max} was found to be 245nm.

3.5.15 Construction of calibration curve:

The calibration curve of Lemongrass Essential Oil was prepared in ethanol. The obtained calibration curves of lemongrass essential oil obeyed the Beer-Lambert's law in the selected concentration.

Table 3.8 Calibration Curve of Lemongrass Essential Oil (Formulation 2)

S. No.	Concentration (µg/ml)	Absorbance
1.	2	0.177
2.	4	0.371
3.	6	0.568
4.	8	0.774
5.	10	0.981

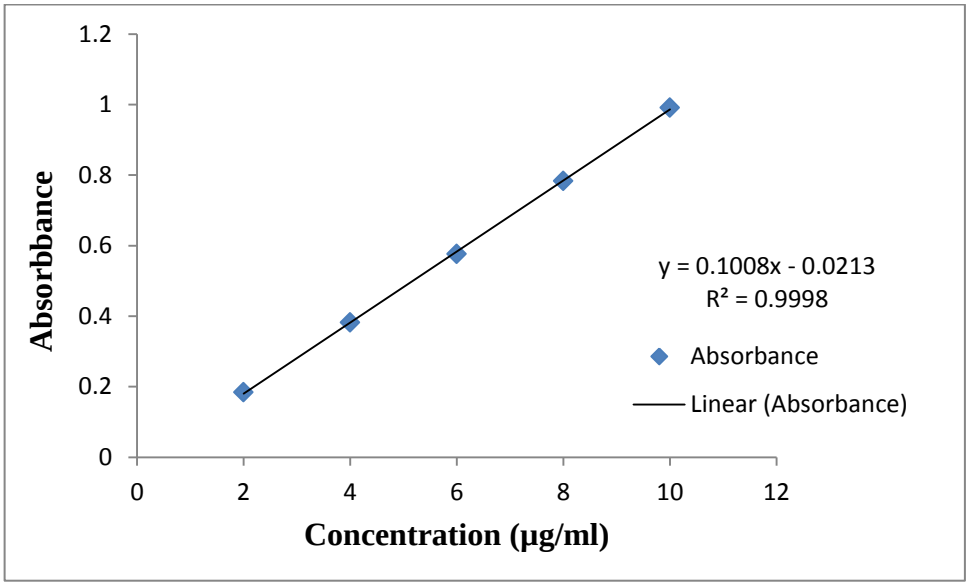

Fig 3.8 Standard Curve of Lemongrass Essential Oil at λ 245nm (Formulation 2)

3.5.16 Calibration Curve for Lemongrass Essential Oil (Formulation 3):

3.5.17 λ_{max} determination:

The λ_{max} was determined by using the U.V. Spectrophotometer. The λ_{max} was found to be 245nm.

3.5.18 Construction of calibration curve:

The calibration curve of Lemongrass Essential Oil was prepared in ethanol. The obtained calibration curves of lemongrass essential oil obeyed the Beer-Lambert's law in the selected concentration.

Table 3.9: Calibration Curve of Lemongrass Essential Oil (Formulation 3)

S. No.	Concentration (µg/ml)	Absorbance
1.	2	0.184
2.	4	0.382
3.	6	0.576
4.	8	0.783
5.	10	0.991

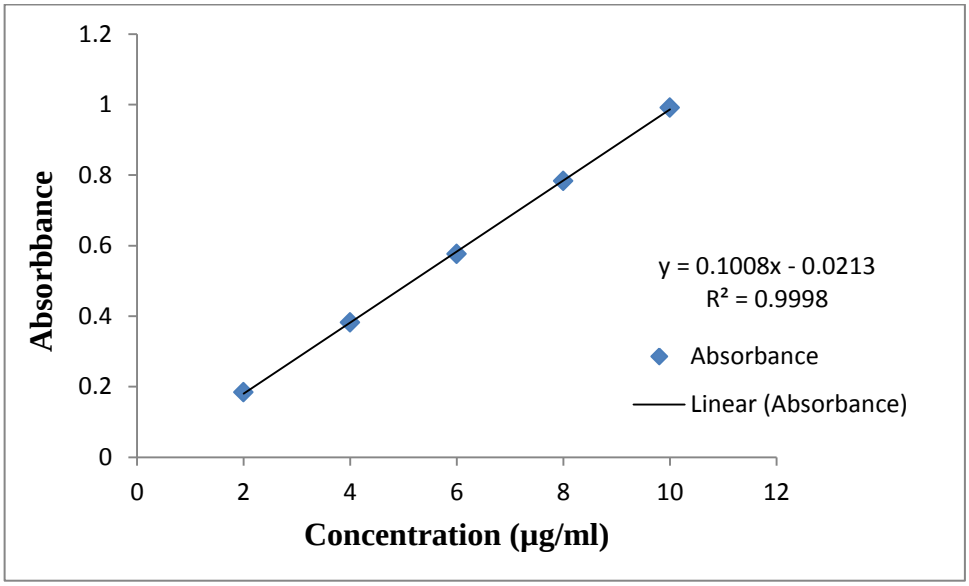

Fig 3.9 Standard Curve of Lemongrass Essential Oil at λ 245nm (Formulation 3)

3.6 Drug Content:

The drug content of the hand sanitizer was determined by dissolving an accurately weighed quantity 1000mg in 100ml of solvent (Ethanol).

The solutions were kept for shaking for 4 hours and kept for 6 hours for complete dissolution of the formulations. Then the solutions were filtered. Then 2ml of this solution was diluted again into 100ml of solvent (ethanol). Then the serial dilutions were made for example 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml.

Table 3.10 Percent Drug Content of Formulation (Eucalyptus Oil)

S.No.	Formulation	% Drug content
1.	F1	89.8%
2.	F2	93%
3.	F3	97.4%

Table 3.11 Percent Drug Content of Formulation (Lemongrass Oil)

S.No.	Formulation	% Drug content
1.	F1	87%
2.	F2	88%
3.	F3	90%

3.7 In-vitro drug release study:

In-vitro drug release studies of formulation of alcohol based herbal hand sanitizer were carried out to estimate the amount of drug that is able to cross the biological membrane. A comparative in-vitro release profile through dialysis membrane from sanitizer is present in Figure 3.10. The data from the release profile is present in Table 3.12.

Table 3.12 Percent of drug release from hand sanitizer formulations at different time intervals

Time	F1	F2	F3
0	0.00	0.00	0.00
0.5	8.1±1.20	7.4±0.62	9.1±1.23
1	11.2±1.44	9.3±0.68	12.0±1.64
2	13.2±0.84	11.3±0.86	14.0±1.21
4	16.0±0.99	13.2±0.82	20.1±1.04
6	26.4±1.22	19.4±0.87	31.2±1.20
8	44.1±1.20	27.0±1.05	47.3±1.08
24	52.6±1.40	35.2±1.42	62.6±1.46

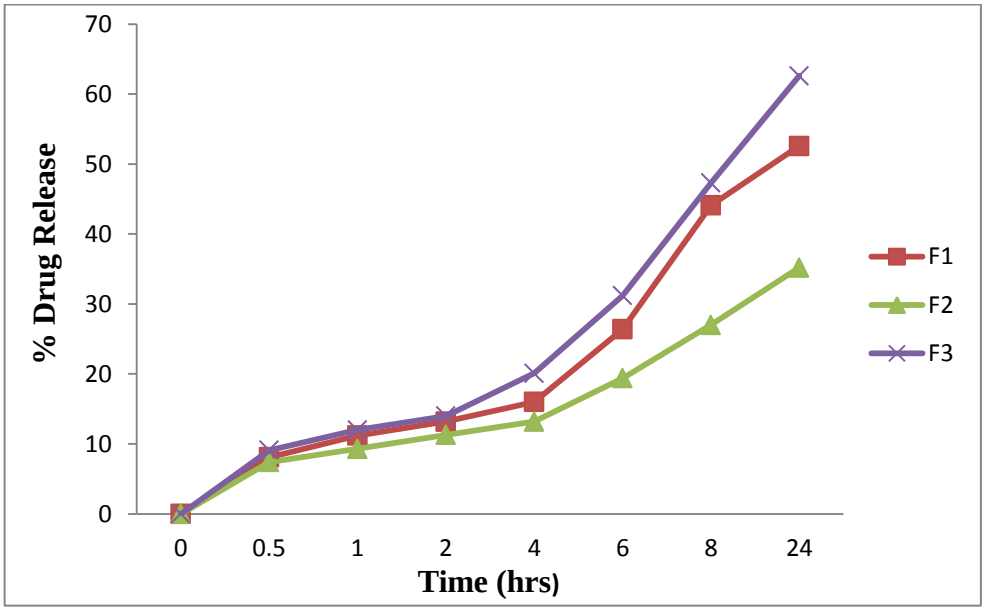

Fig 3.10 Drug release % of alcohol based herbal hand sanitizer

3.8 Antimicrobial study:

In order to evaluate the antibacterial activity of the manufactured hand sanitizer formulations, a total of three bacterial species were chosen. Utilizing the disc diffusion technique, the antibacterial efficacy of produced formulations was assessed (as mentioned before). the outcomes of the incubation in terms of the zone of inhibition against, some micro-organisms are quoted in Table 3.13.

Table 3.13: Antimicrobial study results

Name of the Micro-organisms	**Zone of inhibition (diameter in mm)**			
	F1	**F2**	**F3**	**Std. (Streptomycin)**
Escherichia coli	24	27	31	33
Bacillus subtilis	22	26	29	32
Staphylococcus aureus	23	25	28	31

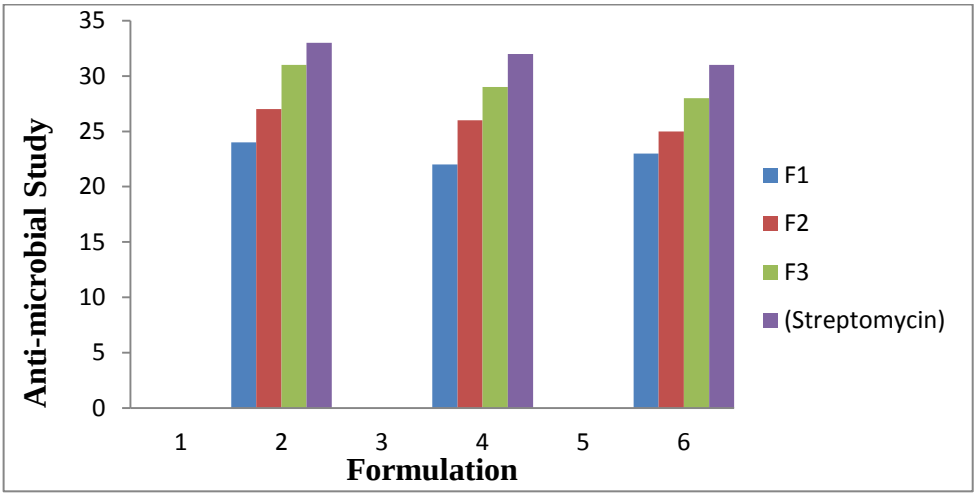

Fig 3.11 Antimicrobial study

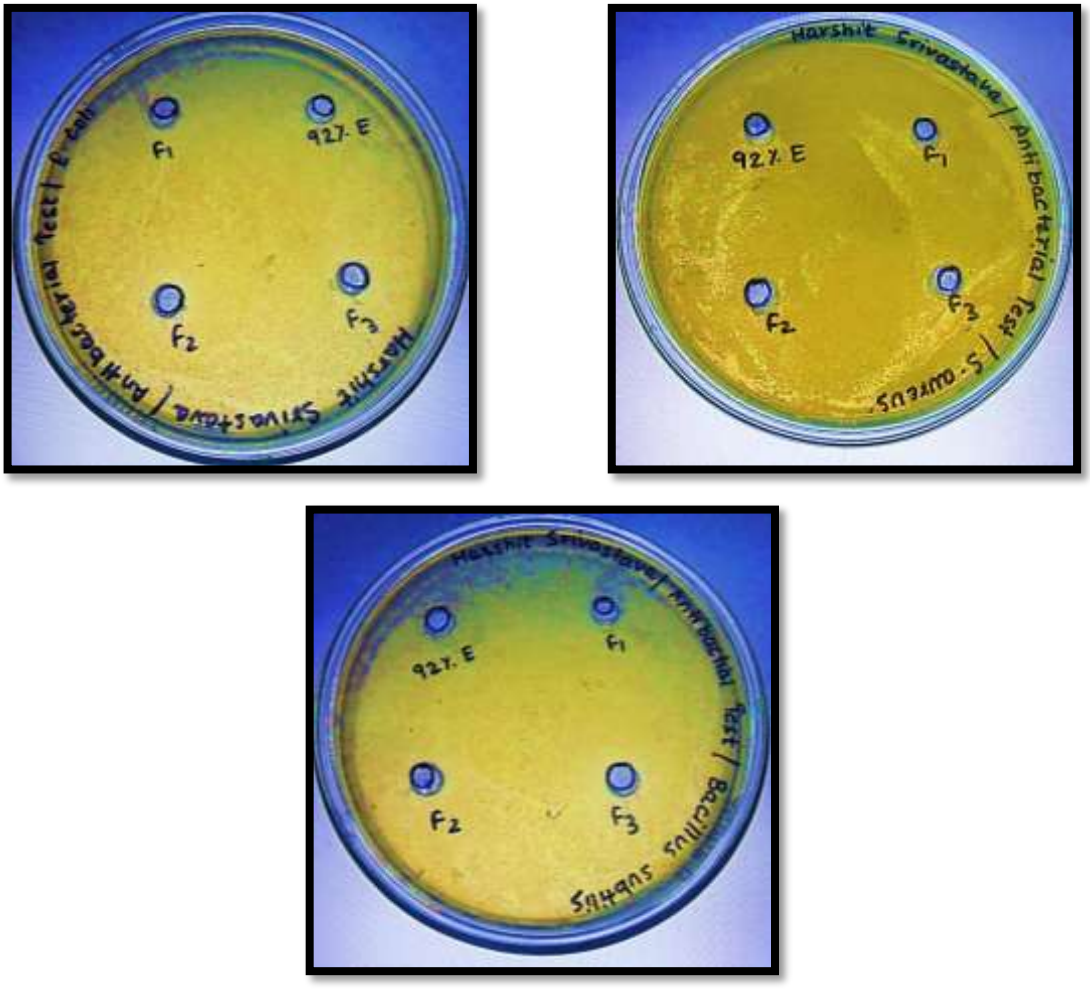

Fig 3.12 Result of Antimicrobial studies

3.9 Stability study:

Table 3.14 Effect of different storage condition on percent entrapment

Time (Months)	**Percent Entrapment (5±3˚C)**	**Percent Entrapment (25±2˚C)**
0	70.2±0.768	72.02±0.768
1	69.1±0.450	71.11±0.363
2	68.5±0.213	67.8±0.452
3	69.4±0.361	68.52±0.532

The prepared alcohol based herbal hand sanitizer containing eucalyptus oil, lemongrass oil, clove oil and aloe vera gel were found to be stable upon storage for 3 months, where no changes was observed in their physical appearance, pH and drug release. Data is present in table 3.14.

4. CONCLUSION:

The present work was carried out to develop a novel alcohol based herbal hand sanitizer. It is a safe, effective, beneficial and overall chemical free way of stopping transmission of diseases. The alcohol based herbal hand sanitizer containing eucalyptus oil, lemongrass oil, clove oil and aloe vera gel which shows various antibacterial, antifungal and antiseptic activities, which helps in treating bacterial diseases and remove pathogens to maintain sanitization. This Methodology adopted for preparation of alcohol based herbal hand sanitizer was very simple and cost effective. The three formulations were prepared, which was named as F1, F2 and F3. From the various Anti-microbial studies performed, the prepared F3 formulation is very effective for bacterial diseases problems.

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CONFLICT OF INTEREST:

The authors have no conflict of interest.

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